Bayer Schering Pharma

Report No.: AT06222

PES Vorstufe 2342

SALMONELLA/MICROSOME TEST
PLATE INCORPORATION AND PREINCUBATION METHOD

Report of study T 5081015
BY
MRS. M. NERN

PERFORMING LABORATORY:

Bayer Schering Pharma AG
GDD-GED-TOX-IT Genetic Toxicology
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SPONSOR:

Bayer MaterialScience AG
51368 Leverkusen
Germany

Study Completion Date: 05 APR 2011

GLP Compliance Statement

Test Item : PES Vorstufe 2342
Study No. : T 5081015

Except for the following deviation the study was conducted in compliance with the OECD Principles of Good Laboratory Practice as revised in 1997 [ENV/MC/CHEM(98)17] and with the revised German Principles of Good Laboratory Practice according to Annex I German Chemicals Act (Bundesgesetzblatt, Volume 2008, Part I, No 28, 1173-1184, issued July 11, 2008). The deviation was as follows: No analytical data were submitted by the sponsor concerning the chemical composition of the test item. However, this deviation did not limit the assessment of the results.

M. Nern

Mrs. M. Nern

Wuppertal, 16 MAR 2011

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Quality Assurance Statement

Study No.: T5081015

Test Item: PES Vorstufe 2342

On the dates given below inspections were conducted by the Quality Assurance to ensure that no deviations exist that are likely to affect the integrity of this study.
The Quality Assurance Unit monitors the conduct of each study by study-based inspections or by process-based inspections of a similar type of study if the short-term nature of a study precludes inspection while it is in progress. Routine procedures and the equipment used in the relevant laboratory areas are inspected regularly and reports are made in accordance with current SOPs.

*(study plan amendments, if any, were duly audited and reported to the Study Director and Management)

Date of Audits / Inspections	Phases Audited / Inspected		Date of Report to Study Director and Management
Jun-11-2010	Study Plan *		Jun-11-2010
Mar-11-2011	process based	Administration / Dosing, Raw Data / Documentation	Mar-11-2011
Mar-28-2011	Main Report	1. Draft	Mar-28-2011
Apr-01-2011	Main Report	Final Draft	Apr-01-2011

The results of this study including the methods used have been checked on the basis of the current SOPs.
They have been correctly reported and the report reflects the raw data.
In case of a multi-site study audits at the test sites are presented in the QA Statement of the Principal Investigator's report (see appendix).

Quality Assurance Unit
Global R&D Quality, GLP-Mgmt.

Date: Apr-01-2011

Signature: Ursula Turek
Ursula Turek

1. Signatures

M. Nern
Study Director

APR 05, 2011
Date

Dr. T. Steger-Hartmann
Head of Investigational Toxicology

APR 05, 2011
Date

2. Summary

PES Vorstufe 2342 was initially investigated using the Salmonella/microsome plate incorporation test for point mutagenic effects in doses of up to and including 5000 µg per plate on five Salmonella typhimurium LT2 mutants. These comprised the histidine-auxotrophic strains TA 1535, TA 100, TA 1537, TA 98 and TA 102. The independent repeat experiment was performed as preincubation modification for 20 minutes at 37 °C. Other conditions remained unchanged.

Doses up to and including 5000 µg per plate did not cause any bacteriotoxic effects. Substance precipitation occurred in the plate incorporation test at the dose of 1581 µg per plate and above and in the preincubation assay at the dose of 5000 µg per plate with S9 mix.

Evidence of mutagenic activity of PES Vorstufe 2342 was not seen. No biologically relevant increase in the mutant count, in comparison to the negative controls, was observed in any of the strains tested, without and with S9 mix, in the plate incorporation as well as in the preincubation modification, under the experimental conditions applied.

The positive controls sodium azide, nitrofurantoin, 4-nitro-1,2-phenylene diamine, mitomycin C, cumene hydroperoxide and 2-aminoanthracene had a marked mutagenic effect, as was seen by a biologically relevant increase in mutant colonies compared to the corresponding negative controls.

Therefore, PES Vorstufe 2342 is considered to be non-mutagenic in the Salmonella/microsome test.

3. Introduction

The evaluation of the mutagenicity of the test substance was performed using the Salmonella/microsome test, also termed the Ames test, as described by Ames et al. (1973a, 1975) and Maron and Ames (1983).

The Salmonella/microsome test is a screening method which detects point mutation caused by chemical agents in vitro. Auxotrophic mutants of Salmonella typhimurium are used to demonstrate this effect. For this purpose, the rate of reversion to prototrophy is evaluated in negative control and treated groups. A mutagenic effect is assumed if this rate increases sufficiently in the treated groups.

Mammalian metabolism, which is of great significance in chemical mutagenesis, is simulated in this test by the 9000 g fraction of homogenized mammalian livers. Together with co-factors, this forms the "S9 mix" which represents the metabolic model in this test.

The method itself is considered to be very sensitive (Herbold et al., 1976; Herbold, 1978) and is well suited for fast screening. Available literature indicates a high correlation between the positive and negative responses of the Ames assay and the carcinogenic activity of the tested substances (McCann et al., 1975a, 1976; Purchase et al., 1976, 1978). In addition, the test represents a good screening system for potential carcinogenic effects, although the results should not be overrated, as this high correlation may not apply to all substance groups (Ames, 1979; Andrews et al., 1978; Clayson, 1980; Glatt et al., 1979 and Rinkus and Legator, 1979; Zeiger, 1987).

Study Initiation Date : 01 JUN 2010
Experimental Starting Date : 24 FEB 2011
Study Start Date : see Experimental Starting Date
Experimental Completion Date : 14 MAR 2011

The study plan, raw data, a retention sample of the test item and the final report are retained in the archives specified by the test facility Toxicology of Bayer Schering Pharma AG in Wuppertal.

4. Material and Methods

4.1. Substances

4.1.1. Test substance

Name of test substance	:	PES Vorstufe 2342
Batch number	:	LB06603520
Content	:	estimated 100 % (indicated by the sponsor)
Approved	:	until 22 OCT 2010 (identity dated 25 MAY 2010) until 21 MAR 2011 (Check of expiry date,11 OCT 2010)
Visual appearance[1]	:	colorless, viscous liquid
Storage	:	refrigerator, dark
Chemical name	:	Castor Oil, reaction product with Soybean Oil
Structure	:	not indicated by the sponsor
EC-No.	:	919-697-6
Indication	:	binder for coating material and adhesive

A stability test in the solvent[2] did not reveal significant degradation of the active ingredient.

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[1] does not reflect chemical composition
[2] solvent will be used as technical term, even if the formulation is a suspension or a emulsion

4.1.2. Positive Controls

Sodium azide (Na-azide, Fluka), order no. 71289, batch no. 1370115/32208002 a direct-acting mutagen used as specific positive control for TA 1535.

Nitrofurantoin (NF, SIGMA), order no. N-7878, lot no. 067K0737, a direct-acting mutagen used as specific positive control for TA 100.

4-Nitro-1,2-phenylene diamine (4-NPDA, Merck-Schuchardt), order no. 806294, batch no. S5061894810, a direct-acting mutagen used as specific positive control for TA 1537 and TA 98.

Mitomycin C (MMC, Fluka), order no. 69824, lot & filling number 1322253/32207047, a direct-acting mutagen used as specific positive control for TA 102 in plate incorporation trials.

Cumene hydroperoxide (Cumene, Sigma-Aldrich), order no. 24,750-2, lot no. S20365-215, a direct-acting mutagen used as specific positive control for TA 102 in preincubation trials.

2-Aminoanthracene (2-AA, Fluka), order no. 06765, batch no. 455186/1 12608036, a promutagen which reverts all the strains and serves as a control for the activating effect of the S9 mix.

The positive controls sodium azide, nitrofurantoin, 4-nitro-1,2-phenylene diamine, mitomycin C and cumene hydroperoxide were only used without S9 mix; the positive control 2-aminoanthracene was only used with S9 mix.

4.2. Indicator Organisms

4.2.1. Description of Test Strains

Histidine-deficient mutants of *Salmonella typhimurium* LT2 served as indicators to demonstrate point mutagenic effects. These strains were selected specifically for the Salmonella/ microsome test. Since point mutations can be divided into two basic classes, base-pair substitutions and frameshift mutations, several strains were used which cover both types.

These included the strains selected by Ames et al. (1973b), Salmonella typhimurium TA 1535 and TA 1537, as well as Salmonella typhimurium TA 100, TA 98 and TA 102 developed by McCann et al. (1975b) and Levin et al. (1982), respectively. TA 1535 and TA 100 bear the base-pair substitution, his G 46, and TA 100 additionally contains the plasmid pKM 101. This R factor also contained in TA 98 and TA 102, codes for an ampicillin resistance and should raise the sensitivity of the strains. TA 102 carries the ochre mutation his G 428 on the multicopy plasmid pAQ1, which codes in addition for tetracycline resistance. TA 1537 and TA 98 bear frameshift markers. TA 1537 exhibits the +1 mutant, his C 3076, while TA 98 bears the +2 type, his D 3052.

Furthermore, the strains have other properties, which should increase their sensitivity. They are all deep rough, i.e. partly deficient in lipopolysaccharide side chains in their cell walls, enabling larger molecules to penetrate the bacterial cell wall and produce mutations. With the exception of TA 102, all strains have reduced capability to repair DNA-damage which increases the likelihood that such damage results in mutations.

Whereas TA 1535 was used in addition to TA 100, TA 1538 is not normally used in addition to TA 98. This has two reasons:

1. There is no relevant increase in the spontaneous mutant counts of TA 98, compared to the spontaneous range of TA 1538. Special differences in sensitivity existing between TA 1535 and TA 100, and attributed to the relatively high spontaneous rate of TA 100 (10 times that of TA 1535), do not exist between TA 1538 and TA 98.

2. An international general inquiry had shown that using TA 1538 in addition to any of the test strains in this study would not provide further information of biological relevance (Herbold, 1983).

This is in agreement with international guidelines, as published by OECD, EEC, or EPA. Strain TA 1538 was either deleted in these guidelines, or never introduced at all. Maron and Ames (1983) also reported: "Although TA 1538 is useful for the detection of particular aromatic frameshift mutagens such as 4-nitro-o-phenylene diamine, we decided to drop the strain because it overlaps considerably with TA 98".

TA 1538, which differs from TA 98 in lacking the plasmid pKM 101, is used in spite of these considerations, if questionable TA 98-results need clarification. This was not the case in the present investigation, however.

4.2.2. Origin of Strains

The original strains were obtained from Prof. Bruce Ames and arrived at Toxicology, Bayer Schering Pharma AG Wuppertal, on 15 AUG 1997.

4.2.3. Production of Stock Cultures

Immediately upon receipt, the samples were inoculated on nutrient agar plates, to which ampicillin had been added for the TA 100, TA 98 and TA 102 strains. The plates for TA 102 contained additionally tetracycline. These plates were incubated at 37 °C for approximately 24 hours. Samples were taken from individual colonies with a sterile inoculation loop, and transferred to nutrient broth[3]. In the case of TA 100, TA 98 and TA 102 ampicillin had also been added to this broth. The broth for TA 102 contained additionally tetracycline. The samples were again incubated overnight at 37 °C. New samples of these cultures were inoculated onto nutrient agar plates, which had again been provided with ampicillin for TA 100, TA 98 and TA 102 and in addition with tetracycline for TA 102.

After an incubation period of approximately 24 hours at 37 °C, new samples of individual colonies from these plates were transferred to flasks containing approximately 20 ml normal nutrient broth. This inoculum was incubated overnight at 37 °C, after which a small sample was taken to check the genotype. At the same time, the remaining cultures were treated with DMSO to protect against the effects of freezing, and immediately frozen at -80 °C in 1 ml portions (Ames et al., 1973b; McCann et al., 1975b). No ampicillin- or tetracycline-resistance test was done on the samples used for testing genotype since the cultures had already been sufficiently selected by ampicillin and tetracycline.

[3] if not otherwise indicated Oxoid No. 2 was used

In addition to the test for crystal-violet sensitivity (deep rough character), a test was done for UV sensitivity (uvrB) where appropriate. The crystal-violet and UV sensitivity tests are described below. The frozen cultures which did not produce satisfactory results here were discarded. Remaining cultures were stored for future tests. In addition, frozen cultures of batches with unsatisfactory negative and/or positive control results in the definitive tests were also discarded.

Whenever new stock cultures needed to be produced, individual cultures grown on nutrient agar were used, to which ampicillin had been added for the TA 100, TA 98 strains and to which ampicillin and tetracycline was added for strain TA 102. Samples of these individual colonies were then transferred to approximately 20 ml nutrient broth, incubated, divided up, and checked for crystal-violet and UV sensitivity, if appropriate.

One 1 ml-portion was thawed for each test and strain, and quantities of 0.2 ml of the thawed culture were added to 10 ml nutrient broth. This culture was incubated overnight at 37 °C and used only on the same day. A new, small stock culture, which had been checked for its properties directly before freezing, was thus available for each individual test. In general this obviated any need to re-check the genotype for each Salmonella/microsome test. This procedure is in accordance with the methods described by Ames et al. (1975) and Maron and Ames (1983).

4.2.4. Checking the Genotype of Stock Cultures

4.2.4.1. Histidine Requirement

A special test for histidine requirement was not necessary since histidine dependence of the cultures was automatically checked by the accompanying negative controls in each individual test of this study. The number of mutants per individual plate is listed in the Tables 1 to 10.

4.2.4.2. Ampicillin Resistance (pKM 101)

A special test for ampicillin resistance was not necessary since strains TA 100, TA 98 and TA 102 were incubated on ampicillin containing nutrient agar and formed individual colonies. Consequently surviving bacteria were ampicillin resistant.

4.2.4.3. Tetracycline Resistance (pAQ1)

A special test for tetracycline resistance was not necessary since TA 102 was incubated on nutrient agar containing in addition to ampicillin tetracycline and formed individual colonies. Consequently surviving bacteria were also tetracycline resistant.

4.2.4.4. Crystal-Violet Sensitivity (deep rough)

A quantity of 0.1 ml was taken from the samples of individual stocks and spread onto nutrient agar, using four plates per strain. After a few minutes, filter papers, to which 10 µl of an aqueous, crystal-violet solution had been added at a concentration of 1 mg/ml, were placed in the middle of the plates. The plates were then incubated overnight at 37 °C. The diameters of the inhibition zones that had formed were then measured. The inhibition zones of all stock batches used indicated an adequate sensitivity to crystal-violet.

4.2.4.5. UV Sensitivity (uvrB)

As described under 4.2.4.4, samples were spread onto nutrient agar plates. One half of each plate was covered with aluminum foil and irradiated without a lid for six seconds (TA 1535 and TA 1537) or eight seconds (TA 100 and TA 98) with UV light of a wavelength of 254 nm at a distance of 33 cm. The irradiated plates were incubated as described under 4.2.4.4 and checked. To demonstrate adequate sensitivity in this test, cultures had to show an inhibition of growth over half their area, i.e. no bacteria should have grown on the irradiated half. This was the case with all the stock batches used. For TA 102 no such test is needed.

4.2.5. Stock Batches

Stock Batches Used in Tables		Strain
1-5	6-10	
15.12.10/4	15.12.10/3	TA 1535
15.12.10/3	15.12.10/3	TA 100
15.12.10/3	15.12.10/4	TA 1537
15.12.10/4	15.12.10/2	TA 98
15.12.09/3	15.12.09/2	TA 102

4.3. S9 Mix

S9 mix was used to simulate the mammalian metabolism of the test substance. It was made from the livers of at least six adult male Sprague Dawley rats, of approximately 200 to 300 g in weight. For enzyme induction, the animals received a single intraperitoneal injection of Aroclor 1254, dissolved in corn oil, at a dose of 500 mg/kg body weight, five days prior to sacrifice. The animals were prepared unfasted, following the directions of Ames et al. (1975) and Maron and Ames (1983).

The rats were terminated. Livers were removed under sterile conditions immediately after sacrifice and kept at 4 °C until all animals had been prepared. All the remaining steps were carried out under sterile conditions at 4 °C.

The livers were washed with cold (4 °C), 0.15 M KCl solution (approximately 1 ml KCl per 1 g liver), and then homogenized in fresh, cold (4 °C), 0.15 M KCl (approximately 3 ml KCl per 1 g liver). The homogenate was then centrifuged in a cooling centrifuge at 4 °C and 9000 g for 10 minutes. The supernatant (the S9 fraction) was stored at -80 °C in small portions.

These portions were slowly thawed before use. The S9 mix was freshly prepared (Ames et al., 1973a), kept on ice and used only on the same day.

Seventy ml of cofactor solution are composed as follows:

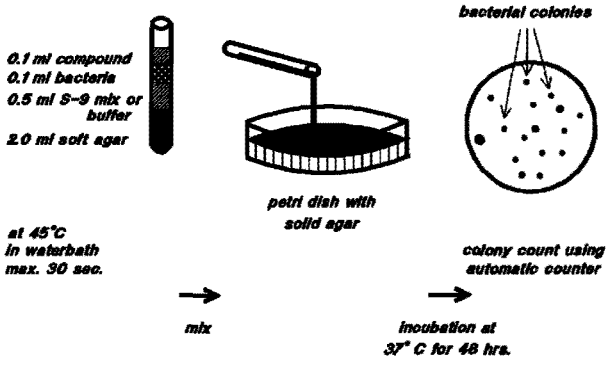
$MgCl_2 \times 6\ H_2O$	162.6 mg
KCl	246.0 mg
Glucose-6-phosphate, disodium salt	179.1 mg
NADP, disodium salt	315.0 mg
Phosphate buffer	100.0 mM

The S9 mix comprised 10 % S9 fraction, 70 % cofactor solution and 20 % 0.15 M KCl. The S9 fraction was derived from the preparation dated January 26, 2009 (protein content 24.4 mg per ml). Prior to first use, each batch was checked for its metabolizing capacity by using reference mutagens; appropriate activity was demonstrated. At the beginning of each experiment 4 aliquots of the S9 mix were plated (0.5 ml per plate) in order to assess its sterility. This was repeated after completion of test tube plating. The sterility control plates were then incubated for 48 hours at 37 °C. No indication of contamination of S9 mix was found.

4.4. Test Design

The initial plate incorporation test followed the directions of Ames et al. (1973a, 1975) and Maron and Ames (1983).

Plate Incorporation Test



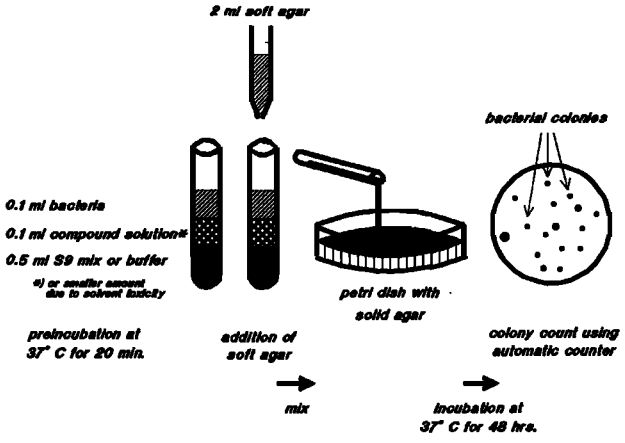
For the mutant count, three plates were used, both with and without S9 mix, for each strain and dose. An equal number of plates, filled with the solvent minus the test substance, comprised the negative control. Each positive control also contained three plates per strain. In experiments without S9 mix buffer was used as replacement.

Generally, the amount of solvent for the test substance and for the controls is 0.1 ml per plate. However, in the present study the amount of solvent per plate used for the dose of PES Vorstufe 2342 at 50 µg per plate was reduced to 0.01 ml per plate due to the range of the stability test with PES Vorstufe 2342. The lacking volume of 0.09 ml solvent was added directly to the tubes.

The doses for the first trial were routinely determined on the basis of a standard protocol: if not limited by solubility 5000 µg or 5 µl per plate were used as the highest dose. At least four additional doses were routinely used. If less than three doses were used for assessment, at least two repeats were performed. The results of the first experiment were then considered as a pre-test for toxicity. However, in case of a positive response or if at least three doses could be used for assessment, the first trial was included in the assessment. If the second test confirmed the results of the first, no additional repeat was performed. Doses of repeats were chosen on the basis of the results obtained in the first experiment.

The independent repeat was performed as preincubation in a water bath at 37 °C for 20 minutes. At the end of the preincubation period 2 ml of molten soft agar were added to the tubes, the content mixed and plated.

Preincubation Test



For the mutant count, three plates were used for each strain and dose. An equal number of plates, filled with the solvent minus the test substance, comprised the negative control. Each positive control also contained three plates per strain.

The doses of this trial were determined on the basis of the results of the plate incorporation assay. Doses are given as µg/tube for better separation of plate incorporation and preincubation trials, despite the fact that µg/plate and µg/tube could be used synonymously.

The toxicity of the substance was assessed in two ways. The first method was a gross appraisal of background growth on the plates for mutant determination. If a reduction in background growth was observed, it was indicated in the tables by the letter "b" after the mutant count. Where only a single "b", without any other values, is noted for a concentration, this "b" represents three plates with reduced background growth. (The same applies to the signs "c", "v", "p", "n" or "%", which may also be used in the tables). Secondly, a toxic effect of the substance was assumed when there was a marked and dose-dependent reduction in the mutant count per plate, compared to the negative controls.

The bacterial suspensions were obtained from 17-hour cultures in nutrient broth, which had been incubated at 37 °C and 90 rpm. These suspensions were used for the determination of mutant counts. No standardized procedure was employed to set the bacterial suspensions at a defined density of viable cells per milliliter, since the chosen method of incubation normally produces the desired density. However, the numbers of viable cells were established in a parallel procedure by determining the titers of the negative controls.

The dilution of bacterial suspensions used for the determination of titers was 1:1,000,000. Titers were determined under the same conditions as were the mutations, except that the histidine concentration in the soft agar was increased five-fold to permit the complete growth of bacteria. Total bacterial counts were taken with S9 mix on two plates for each strain. However, if an evaluation was performed only without S9 mix, the bacterial count was taken without S9 mix. The results of these determinations may be seen in the negative controls in Tables 1 to 10.

The tests were performed both with and without S9 mix. Full details are given in the Tables 1 to 10.

The count was made after the plates had been incubated for 48 hours at 37 °C. If no immediate count was possible, plates were temporarily stored in a refrigerator. If not interfered e.g. by precipitation on the plates or coloration of the plates, colonies were counted automatically using the Petri Viewer Mk2 (Sorcerer) of Perceptive Instruments Ltd. Data were transferred to a Windows 2000 (SP4) based client (Compac Computer Corporation Evo D310), which is connected to a server (Linux 4 Server, Red Hat Enterprise based, system number 5-1134). Data were processed Oracle based with the following released Perceptive Instruments Ltd. applications:

- Ames Study Manager 1.21
- Ames Report Generator 1.1
- Sorcerer 2.2
- Change Password Utility 1.0
- Password Management/User Administration 1.0

The following criteria determined the acceptance of an assay:

1. The negative controls had to be within the expected range, as defined by published data (e.g. Maron and Ames, 1983) and/ or the laboratories' own historical data (see Chapter 8).

2. The positive controls had to show sufficient effects, as defined by the laboratories' experience (see Chapter 8).

3. Titer determinations had to demonstrate sufficient bacterial density in the suspension.

Only trials which complied with all three of the above criteria were accepted for assessment. Even if the criteria for points (2.) and (3.) were not met, a trial was accepted if it showed mutagenic activity of the test compound. Furthermore, an unacceptable trial would have been repeated.

The following doses of PES Vorstufe 2342 were evaluated in the test:

	µg per plate	
Negative control	0	
PES Vorstufe 2342	5000	
PES Vorstufe 2342	1581	
PES Vorstufe 2342	500	
PES Vorstufe 2342	158	
PES Vorstufe 2342	50	
Positive control, Na-azide	10	(only TA 1535)
Positive control, NF	0.2	(only TA 100)
Positive control, 4-NPDA	10	(only TA 1537)
Positive control, 4-NPDA	0.5	(only TA 98)
Positive control, MMC	0.2	(only TA 102[4])
Positive control, Cumene	50	(only TA 102[5])
Positive control, 2-AA	3	

[4] only in plate incorporation trials
[5] only in preincubation trials

PES Vorstufe 2342 was dissolved in DMSO (dried with a molecular sieve, 0.3 nm) and formed colorless clear solutions. Mitomycin C was dissolved in deionized water. The other positive controls were dissolved in DMSO.

The solvent used was chosen out of the following solvents, in the order given: water, DMSO, methanol, ethanol, acetone, ethylene glycol dimethylether (EGDE), and DMF according to information given by the sponsor. The order of these solvents is based on their bacteriotoxic effects in preincubation experiments.

No "untreated" negative control was set up for the used solvent, since sufficient evidence was available in the literature (e.g. Maron and Ames, 1983) and from our own experience (see Chapter 8), indicating that this solvent had no influence on the spontaneous mutant counts of the used strains.

4.5. Assessment Criteria

A reproducible and dose-related increase in mutant counts of at least one strain is considered to be a positive result. For TA 1535, TA 100 and TA 98 this increase should be about twice that of negative controls, whereas for TA 1537, at least a threefold increase should be reached. For TA 102 an increase of about 100 mutants should be reached. Otherwise, the result is evaluated as negative. However, these guidelines may be overruled by good scientific judgment.

In case of questionable results, investigations should continue, possibly with modifications, until a final evaluation is possible.

4.6. Study Guidelines

The study was performed according at least to the following guidelines:

Council Regulation No. 440/2008 of 30 May 2008
Official Journal of the European Communities of May 31, 2008,
L 142/248 – L142/255
B.13/14. Mutagenicity - Reverse Mutation Test using Bacteria

OECD Guidelines for Testing of Chemicals No. 471
"Bacterial Reverse Mutation Test"
Adopted: 21st July 1997

Health Effects Test Guidelines; United States Environmental Protection Agency; Prevention, Pesticides and Toxic Substances (7101); EPA712-C-98-247, August 1998
OPPTS 870.5100 - Bacterial Reverse Mutation Test

4.7. Study Identification and Responsibilities

4.7.1. Type of Test and Study Number

Salmonella/Microsome Test: T 5081015

4.7.2. Responsibilities

Head of Toxicology	: Dr. F.-W. Jekat
Head of Investigational Toxicology	: Dr. T. Steger Hartmann
Study Director	: Mrs. M. Nern
Senior Technician	: Mrs. M. Bönning
Head of Archives	: Mrs. Zils
Quality Assurance	: Dr. A. Paeßens
Analyst(s)	: Mrs. Maria Teresa Garcia-Sanchez, Mr. M. Neuland

5. Results

5.1. Description of Results

The colony numbers of each plate and mean values of the assay are listed for each dose in Tables 1 to 10. As may be seen, there was no indication of a bacteriotoxic effect of PES Vorstufe 2342 at doses of up to and including 5000 µg per plate. The total bacteria counts consistently produced results comparable to the negative controls, or differed only insignificantly. Substance precipitation occurred in the plate incorporation test at the dose of 1581 µg per plate and above and in the preincubation assay at the dose of 5000 µg per plate with S9 mix.

None of the five strains used showed a dose-related and biologically relevant increase in mutant counts over those of the negative controls. This applied both to the tests with and without S9 mix (Tables 1 to 5) and was confirmed by the results of the preincubation trials (Tables 6 to 10).

Summary of the Results with PES Vorstufe 2342
in the Salmonella/Microsome Test

S9 mix	TA 1535	TA 100	TA 1537	TA 98	TA 102
without	-ve	-ve	-ve	-ve	-ve
with	-ve	-ve	-ve	-ve	-ve

-ve = negative

The positive controls sodium azide, nitrofurantoin, 4-nitro-1,2-phenylene diamine, mitomycin C, cumene hydroperoxide and 2-aminoanthracene increased mutant counts to well over those of the negative controls, and thus demonstrated the system's sensitivity and the activity of the S9 mix.

5.2. Tabulated Summary of Data

Summary of Mean Values Without S9 Mix					
Table and Group	Strain				
	TA 1535	TA 100	TA 1537	TA 98	TA 102
1-5 µg/Plate					
0	9	151	6	23	253
50	7	145	5	24	228
158	7	148	6	24	215
500	7	152	5	21	257
1581	8	125	5	21	225
5000	8	143	5	22	243
Na-azide	859				
NF		458			
4-NPDA			38	84	
MMC					704
6-10 µg/Tube					
0	9	129	7	15	282
50	8	138	7	15	273
158	8	129	7	16	262
500	9	136	6	13	291
1581	8	123	5	17	230
5000	6	121	4	15	248
Na-azide	818				
NF		451			
4-NPDA			33	67	
Cumene					520

Summary of Mean Values With S9 Mix					
Table and Group	Strain				
	TA 1535	TA 100	TA 1537	TA 98	TA 102
1-5 µg/Plate					
0	11	205	10	27	319
50	11	202	9	29	323
158	9	194	8	25	299
500	10	201	9	26	272
1581	9	202	8	26	272
5000	8	209	8	22	292
2-AA	118	3293	425	2311	821
6-10 µg/Tube					
0	9	193	10	20	314
50	9	192	9	18	339
158	8	174	10	22	349
500	8	186	10	25	327
1581	8	168	8	24	335
5000	10	145	7	20	294
2-AA	122	2229	218	1492	610

6. Assessment

The Salmonella/microsome plate incorporation test, employing doses of up to 5000 µg per plate, showed PES Vorstufe 2342 not to produce bacteriotoxic effects. In the plate incorporation test substance precipitation occurred at 1581 µg per plate and above and in the preincubation test the substance only precipitated with S9 mix at 5000 µg per plate.

Evaluation of individual dose groups, with respect to relevant assessment parameters (dose effect, reproducibility) revealed no biologically relevant variations from the respective negative controls.

In spite of the low doses used, positive controls increased the mutant counts to well over those of the negative controls, and thus demonstrated the system's high sensitivity.

Despite this sensitivity, no indications of mutagenic effects of PES Vorstufe 2342 could be found at assessable doses of up to 5000 µg per plate in any of the Salmonella typhimurium strains, without and with metabolic activation, under the experimental conditions applied.

Due to these results PES Vorstufe 2342 has to be regarded as non-mutagenic in the Salmonella/microsome test.

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8. Historical Controls

8.1. Plate Incorporation Method

Summary of Historical Negative and Positive Controls
Using Mean Values Presented as
Medians (Z) and Semi-Q Range (QR)

2000

Compound and S9 Mix		Strain TA 1535 Z	TA 1535 QR	TA 100 Z	TA 100 QR	TA 1537 Z	TA 1537 QR	TA 98 Z	TA 98 QR	TA 102 Z	TA 102 QR
Water	-	9	1	81	11	7	2	25	6	196	32
DMSO	-	9	2	73	17	7	1	23	6	209	30
Ethanol	-	12	-	97	-	6	-	31	-	209	-
Na-azid	-	629	70								
NF	-			226	35						
4-NPDA	-					106	15	173	21		
Cumene	-									352	43
Water	+	11	1	104	14	10	2	37	5	260	41
DMSO	+	10	2	94	20	8	2	32	7	270	34
Ethanol	+	12	-	106	-	13	-	42	-	230	-
2-AA	+	207	38	1468	223	252	88	1524	160	539	66

2001

Compound and S9 Mix		Strain TA 1535 Z	TA 1535 QR	TA 100 Z	TA 100 QR	TA 1537 Z	TA 1537 QR	TA 98 Z	TA 98 QR	TA 102 Z	TA 102 QR
Water	-	9	3	100	22	6	1	39	4	223	42
DMSO	-	9	2	100	13	6	2	34	13	208	35
Acetone	-	8	-	113	-	8	-	38	-	233	-
Na-azid	-	633	76								
NF	-			264	26						
4-NPDA	-					94	13	172	19		
Cumene	-									365	24
Water	+	11	2	119	22	9	2	50	6	283	30
DMSO	+	10	2	125	19	9	2	41	9	267	32
Acetone	+	12	-	129	-	10	-	45	-	317	-
2-AA	+	193	26	1614	97	270	78	1443	67	532	71

Summary of Historical Negative and Positive Controls Using Mean Values Presented as Medians (Z) and Semi-Q Range (QR)

2002

Compound and S9 Mix		Strain TA 1535 Z	TA 1535 QR	TA 100 Z	TA 100 QR	TA 1537 Z	TA 1537 QR	TA 98 Z	TA 98 QR	TA 102 Z	TA 102 QR
Water	-	14	3	130	29	8	3	35	8	222	50
DMSO	-	14	5	136	15	7	2	31	9	216	35
EGDE	-	21	7	146	8	8	2	27	5	262	38
Na-azid	-	735	80								
NF	-			323	30						
4-NPDA	-					88	11	156	24		
Cumene	-									556	92
Water	+	11	3	151	25	10	2	48	14	275	36
DMSO	+	12	4	151	19	8	2	39	12	270	24
EGDE	+	9	3	144	19	7	1	33	6	306	45
2-AA	+	158	30	1506	126	256	60	1283	139	554	64

2003

Compound and S9 Mix		Strain TA 1535 Z	TA 1535 QR	TA 100 Z	TA 100 QR	TA 1537 Z	TA 1537 QR	TA 98 Z	TA 98 QR	TA 102 Z	TA 102 QR
Water	-	14	5	140	20	7	2	28	4	252	44
DMSO	-	14	3	144	13	7	1	24	5	227	28
EGDE	-	16	2	167	-	7	-	26	-	243	-
Ethanol	-	14	-	150	-	8	-	18	-	221	-
Acetone	-	15	-	134	-	9	-	42	-	279	-
Na-azid	-	679	90								
NF	-			358	42						
4-NPDA	-					91	12	157	18		
MMC	-									530	63
Water	+	13	1	177	30	10	2	41	6	281	32
DMSO	+	11	2	169	22	9	2	34	6	294	32
EGDE	+	11	1	184	-	10	-	39	-	280	-
Ethanol	+	25	-	177	-	9	-	38	-	227	-
Acetone	+	10	-	172	-	12	-	31	-	311	-
2-AA	+	170	28	1526	143	286	78	1247	138	721	114

Summary of Historical Negative and Positive Controls Using Mean Values Presented as Medians (Z) and Semi-Q Range (QR)

2004

Compound and S9 Mix		Strain TA 1535 Z	TA 1535 QR	TA 100 Z	TA 100 QR	TA 1537 Z	TA 1537 QR	TA 98 Z	TA 98 QR	TA 102 Z	TA 102 QR
Water	-	16	3	150	12	8	1	24	7	227	13
DMSO	-	16	4	135	15	7	1	26	9	199	14
EGDE	-	16	-	156	-	7	-	26	-	263	-
Ethanol	-	16	-	135	-	7	-	27	-	218	-
Na-azid	-	651	121								
NF	-			339	28						
4-NPDA	-					91	18	153	16		
MMC	-									541	59
Water	+	13	2	158	20	11	2	42	11	255	20
DMSO	+	11	2	153	23	10	2	40	9	255	24
EGDE	+	12	-	191	-	8	-	48	-	301	-
Ethanol	+	20	-	170	-	10	-	40	-	251	-
2-AA	+	150	29	1436	116	259	57	1164	112	573	79

2005

Compound and S9 Mix		Strain TA 1535 Z	TA 1535 QR	TA 100 Z	TA 100 QR	TA 1537 Z	TA 1537 QR	TA 98 Z	TA 98 QR	TA 102 Z	TA 102 QR
Water	-	19	7	162	22	7	1	25	5	223	20
DMSO	-	16	3	142	17	7	1	24	8	214	22
EGDE	-	19	3	152	33	7	1	29	7	254	27
Ethanol	-	11	-	138	-	7	-	21	-	238	-
DMF	-	10	-	125	-	6	-	24	-	212	-
Na-azid	-	652	66								
NF	-			310	33						
4-NPDA	-					89	12	149	17		
MMC	-									518	38
Water	+	13	3	205	41	8	1	41	8	260	44
DMSO	+	11	2	178	22	8	2	44	8	284	30
EGDE	+	16	2	152	17	8	1	43	7	310	31
Ethanol	+	15	-	127	-	11	-	31	-	287	-
DMF	+	10	-	171	-	7	-	51	-	264	-
2-AA	+	168	28	1426	142	185	58	1157	162	718	99

Summary of Historical Negative and Positive Controls Using Mean Values Presented as Medians (Z) and Semi-Q Range (QR)

2006

Compound and S9 Mix		Strain TA 1535 Z	TA 1535 QR	TA 100 Z	TA 100 QR	TA 1537 Z	TA 1537 QR	TA 98 Z	TA 98 QR	TA 102 Z	TA 102 QR
Water	-	17	5	139	22	7	1	18	6	205	18
DMSO	-	15	3	129	18	7	1	25	7	214	24
EGDE	-	20	-	164	-	7	-	37	-	188	-
Na-azid	-	654	101								
NF	-			303	37						
4-NPDA	-					78	8	142	33		
MMC	-									560	222
Water	+	11	2	144	28	8	1	28	4	268	26
DMSO	+	10	2	157	26	8	1	31	7	261	26
EGDE	+	10	-	178	-	8	-	37	-	279	-
2-AA	+	134	29	1359	126	181	71	1017	107	596	105

2007

Compound and S9 Mix		Strain TA 1535 Z	TA 1535 QR	TA 100 Z	TA 100 QR	TA 1537 Z	TA 1537 QR	TA 98 Z	TA 98 QR	TA 102 Z	TA 102 QR
Water	-	16	7	143	19	7	1	24	3	213	13
DMSO	-	13	5	138	22	7	1	21	4	237	33
EGDE	-	14	-	119	-	7	-	27	-	186	-
Na-azid	-	681	127								
NF	-			355	32						
4-NPDA	-					87	9	152	15		
MMC	-									610	72
Water	+	11	1	172	33	8	2	34	4	288	37
DMSO	+	11	2	174	28	9	1	29	5	285	45
EGDE	+	13	-	149	-	10	-	35	-	252	-
2-AA	+	153	33	1490	343	173	51	1079	204	568	105

Summary of Historical Negative and Positive Controls Using Mean Values Presented as Medians (Z) and Semi-Q Range (QR)

2008

Compound and S9 Mix		Strain TA 1535 Z	TA 1535 QR	TA 100 Z	TA 100 QR	TA 1537 Z	TA 1537 QR	TA 98 Z	TA 98 QR	TA 102 Z	TA 102 QR
Water	-	13	7	143	21	6	1	24	3	220	15
DMSO	-	13	3	149	10	8	1	21	5	250	23
Ethanol	-	14	-	145	-	6	-	29	-	285	-
Acetone	-	15	-	108	-	7	-	34	-	225	-
Na-azid	-	916	127								
NF	-			382	30						
4-NPDA	-					87	10	149	16		
MMC	-									818	79
Water	+	11	2	188	9	10	2	33	3	275	35
DMSO	+	11	1	168	20	10	2	35	6	309	27
Ethanol	+	13	-	142	-	7	-	41	-	359	-
Acetone	+	10	-	139	-	9	-	34	-	291	-
2-AA	+	112	22	2183	311	185	77	1619	259	726	74

2009

Compound and S9 Mix		Strain TA 1535 Z	TA 1535 QR	TA 100 Z	TA 100 QR	TA 1537 Z	TA 1537 QR	TA 98 Z	TA 98 QR	TA 102 Z	TA 102 QR
Water	-	9	1	130	16	8	1	23	5	230	16
DMSO	-	8	1	125	14	7	1	25	4	231	27
Ethanol		9	-	114	-	6	-	23	-	217	-
EGDE	-	8	-	116	-	6	-	14	-	250	-
Na-azid	-	884	94								
NF	-			356	34						
4-NPDA	-					37	7	85	10		
MMC	-									828	255
Water	+	11	1	210	19	9	2	33	7	322	26
DMSO	+	10	2	194	21	9	1	33	5	312	22
Ethanol		6	-	112	-	7	-	38	-	279	-
EGDE	+	9	-	182	-	8	-	24	-	316	-
2-AA	+	96	22	2391	261	195	61	2019	289	817	128

8.2. Preincubation Method

Summary of Historical Negative and Positive Controls Using Mean Values Presented as Medians (Z) and Semi-Q Range (QR)

2000

Compound and S9 Mix		Strain TA 1535 Z	TA 1535 QR	TA 100 Z	TA 100 QR	TA 1537 Z	TA 1537 QR	TA 98 Z	TA 98 QR	TA 102 Z	TA 102 QR
Water	-	11	3	135	8	10	2	24	5	239	23
DMSO	-	8	2	80	16	8	2	21	6	237	20
Ethanol	-	9	-	103	-	11	-	44	-	228	-
Na-azid	-	620	72								
NF	-			294	55						
4-NPDA	-					136	14	176	22		
Cumene	-									469	43
Water	+	12	2	148	15	12	2	31	4	297	28
DMSO	+	9	1	90	14	9	2	29	6	296	20
Ethanol	+	13	-	127	-	14	-	51	-	211	-
2-AA	+	178	36	1353	213	239	54	1349	160	466	39

2001

Compound and S9 Mix		Strain TA 1535 Z	TA 1535 QR	TA 100 Z	TA 100 QR	TA 1537 Z	TA 1537 QR	TA 98 Z	TA 98 QR	TA 102 Z	TA 102 QR
Water	-	10	2	121	24	9	1	40	6	269	25
DMSO	-	8	2	107	16	7	1	32	6	229	24
Acetone	-	13	-	120	-	10	-	37	-	271	-
Na-azid	-	614	92								
NF	-			403	44						
4-NPDA	-					121	17	182	20		
Cumene	-									429	48
Water	+	11	1	136	36	10	1	43	9	305	32
DMSO	+	10	2	123	20	9	2	40	6	281	27
Acetone	+	15	-	142	-	14	-	51	-	315	-
2-AA	+	196	28	1627	93	294	46	1442	120	470	47

Summary of Historical Negative and Positive Controls Using Mean Values Presented as Medians (Z) and Semi-Q Range (QR)

2002

Compound and S9 Mix		Strain TA 1535 Z	QR	TA 100 Z	QR	TA 1537 Z	QR	TA 98 Z	QR	TA 102 Z	QR
Water	-	17	5	153	9	8	2	32	13	272	18
DMSO	-	12	3	145	13	8	2	29	6	255	32
EGDE	-	18	4	158	12	9	2	33	7	264	42
Na-azid	-	740	95								
NF	-			447	31						
4-NPDA	-					114	14	165	17		
Cumene	-									480	35
Water	+	15	2	167	20	11	2	45	11	322	19
DMSO	+	11	2	155	16	9	1	38	9	314	38
EGDE	+	12	10	179	20	9	2	46	10	342	35
2-AA	+	170	22	1550	122	316	57	1234	149	509	34

2003

Compound and S9 Mix		Strain TA 1535 Z	QR	TA 100 Z	QR	TA 1537 Z	QR	TA 98 Z	QR	TA 102 Z	QR
Water	-	18	2	154	19	10	3	31	10	246	26
DMSO	-	15	3	137	18	8	1	20	5	236	24
EGDE	-	16	-	161	-	8	-	26	-	262	-
Ethanol	-	14	-	142	-	7	-	23	-	249	-
Acetone	-	20	-	134	-	12	-	33	-	340	-
Na-azid	-	664	88								
NF	-			463	37						
4-NPDA	-					120	18	164	17		
Cumene	-									477	44
Water	+	13	5	173	32	9	1	39	11	303	63
DMSO	+	11	2	159	24	9	2	34	5	297	28
EGDE	+	15	-	213	-	12	-	52	-	288	-
Ethanol	+	21	-	181	-	8	-	39	-	308	-
Acetone	+	9	-	159	-	17	-	26	-	398	-
2-AA	+	187	28	1527	159	345	60	1255	144	613	94

Summary of Historical Negative and Positive Controls Using Mean Values Presented as Medians (Z) and Semi-Q Range (QR)

2004

Compound and S9 Mix		Strain TA 1535 Z	QR	TA 100 Z	QR	TA 1537 Z	QR	TA 98 Z	QR	TA 102 Z	QR
Water	-	18	3	151	9	11	2	25	5	224	14
DMSO	-	15	3	134	10	8	1	24	6	209	19
EGDE	-	14	-	148	-	9	-	21	-	253	-
Ethanol	-	18	-	130	-	6	-	22	-	218	-
Na-azid	-	609	107								
NF	-			460	56						
4-NPDA	-					111	18	157	18		
Cumene	-									470	34
Water	+	14	2	182	10	10	2	35	5	266	11
DMSO	+	12	2	156	24	10	2	36	7	251	25
EGDE	+	13	-	206	-	11	-	51	-	298	-
Ethanol	+	24	-	182	-	9	-	34	-	221	-
2-AA	+	163	14	1515	134	293	25	1145	120	512	56

2005

Compound and S9 Mix		Strain TA 1535 Z	QR	TA 100 Z	QR	TA 1537 Z	QR	TA 98 Z	QR	TA 102 Z	QR
Water	-	18	4	158	15	8	3	29	6	241	38
DMSO	-	17	4	144	18	7	2	27	8	228	19
EGDE	-	15	-	144	-	8	-	23	-	239	-
Ethanol	-	13	-	139	-	8	-	30	-	260	-
DMF	-	12	-	93	-	8	-	32	-	270	-
Na-azid	-	637	72								
NF	-			451	44						
4-NPDA	-					107	21	155	17		
Cumene	-									428	39
Water	+	14	2	205	30	11	2	44	8	289	47
DMSO	+	11	3	183	31	9	2	44	7	289	33
EGDE	+	13	-	193	-	11	-	32	-	308	-
Ethanol	+	21	-	191	-	10	-	29	-	276	-
DMF	+	10	-	124	-	9	-	51	-	284	-
2-AA	+	161	18	1448	159	297	46	1142	97	607	99

Summary of Historical Negative and Positive Controls Using Mean Values Presented as Medians (Z) and Semi-Q Range (QR)

2006

Compound and S9 Mix		Strain TA 1535 Z	QR	TA 100 Z	QR	TA 1537 Z	QR	TA 98 Z	QR	TA 102 Z	QR
Water	-	19	5	148	20	7	1	19	7	242	26
DMSO	-	15	3	130	14	7	1	19	4	217	23
EGDE	-	22	-	151	-	7	-	26	-	274	-
Na-azid	-	668	87								
NF	-			444	33						
4-NPDA	-					98	10	142	10		
Cumene	-									443	26
Water	+	12	3	182	39	9	1	29	6	275	29
DMSO	+	10	2	142	28	9	1	30	5	273	29
EGDE	+	10	-	191	-	8	-	39	-	252	-
2-AA	+	142	25	1397	101	217	50	982	91	528	62

2007

Compound and S9 Mix		Strain TA 1535 Z	QR	TA 100 Z	QR	TA 1537 Z	QR	TA 98 Z	QR	TA 102 Z	QR
Water	-	16	6	157	20	8	2	26	4	256	37
DMSO	-	13	3	137	20	8	1	20	4	237	24
EGDE	-	14	-	160	-	8	-	27	-	284	-
Na-azid	-	683	118								
NF	-			489	48						
4-NPDA	-					107	12	156	13		
Cumene	-									482	35
Water	+	11	2	214	29	9	1	33	4	322	35
DMSO	+	11	2	176	21	9	2	30	4	293	33
EGDE	+	13	-	205	-	8	-	34	-	306	-
2-AA	+	150	22	1473	231	203	54	1007	172	514	42

Summary of Historical Negative and Positive Controls Using Mean Values Presented as Medians (Z) and Semi-Q Range (QR)

2008

Compound and S9 Mix		Strain TA 1535 Z	TA 1535 QR	TA 100 Z	TA 100 QR	TA 1537 Z	TA 1537 QR	TA 98 Z	TA 98 QR	TA 102 Z	TA 102 QR
Water	-	17	6	151	16	9	1	21	6	249	21
DMSO	-	12	5	136	15	8	2	20	4	248	24
Ethanol	-	16	-	160	-	9	-	30	-	311	-
Acetone	-	16	-	145	-	10	-	25	-	216	-
EGDE	-	15	-	148	-	8	-	19	-	281	-
Na-azid	-	869	139								
NF	-			568	39						
4-NPDA	-					107	12	163	16		
Cumene	-									559	52
Water	+	12	3	178	24	11	2	39	6	310	34
DMSO	+	13	2	164	28	11	2	33	5	309	28
Ethanol	+	15	-	159	-	13	-	40	-	353	-
Acetone	+	10	-	143	-	11	-	29	-	248	-
EGDE	+	15	-	182	-	14	-	36	-	322	-
2-AA	+	132	26	2244	283	245	67	1426	246	640	78

2009

Compound and S9 Mix		Strain TA 1535 Z	TA 1535 QR	TA 100 Z	TA 100 QR	TA 1537 Z	TA 1537 QR	TA 98 Z	TA 98 QR	TA 102 Z	TA 102 QR
Water	-	10	2	143	13	9	1	23	4	247	27
DMSO	-	9	1	123	14	7	2	21	4	237	24
Ethanol	-	6	-	122	-	6	-	15	-	227	-
Na-azid	-	914	151								
NF	-			539	44						
4-NPDA	-					41	8	83	13		
Cumene	-									519	55
Water	+	12	1	213	16			31	5	315	28
DMSO	+	10	2	117	26	10	2	30	4	223	33
Ethanol	+	9	-	138	-			31	-	329	-
2-AA	+	112	14	2531	454	260	31	1603	273	704	153

9. Stability in Solvent

Results of the analyses for stability of
PES Vorstufe 2342

nominal value in mg/ml	content as % of start value after storage time in hours	
	0	24
1	100	83
200	100	85

According to these results PES Vorstufe 2342 is stable in the solvent at room temperature at concentrations ranging from 1 mg/ml to 200 mg/ml for at least twenty-four hours, a time interval, which covers the time range from preparation of the formulation to last treatment. Due to the test solution concentration needed for the IR-determination, a formulation with a concentration of 0.01 mg/ml could not be tested. The low concentration was therefore increased to 1 mg/ml.

10. Statement of GLP Compliance

Ministerium für Arbeit, Gesundheit und Soziales
Des Landes Nordrhein-Westfalen

Fürstenwall 25, 40219 Düsseldorf — Aktenzeichen II A 5 - 31.11.46 06

Gute Laborpraxis/Good Laboratory Practice
GLP-Bescheinigung/Statement of GLP Compliance
(gemäß/according to § 19b Abs. 1 Chemikaliengesetz)

Eine GLP-Inspektion zur Überwachung der Einhaltung der GLP-Grundsätze gemäß Chemikaliengesetz bzw. Richtlinie 88/320/EG wurde durchgeführt in

Assessment of conformity with GLP according to Chemikaliengesetz and Directive 88/320/EEC at:

☒ Prüfeinrichtung/Test facility ☐ Prüfstandort/Test site

Bayer HealthCare AG
BSP-GDD-GED
Toxikologie
Aprather Weg 18 a
42096 Wuppertal

Prüfungen nach Kategorien (gemäß ChemVwV-GLP Nr. 5.3/OECD guidance)	Areas of Expertise (according ChemVwV GLP Nr. 5.3/OECD guidance)
Kategorie 1 Prüfungen zur Bestimmung der physikalisch-chemischen Eigenschaften und Gehaltsbestimmungen	**category 1** physical-chemical testing
Kategorie 2 Prüfungen zur Bestimmung der toxikologischen Eigenschaften	**category 2** toxicity studies
Kategorie 3 Prüfungen zur Bestimmung der erbgutverändernden Eigenschaften (in vitro und in vivo)	**category 3** mutagenicity studies
Kategorie 9 Biochemische Toxikologie; Kurzzeitkanzerogenese; Immuntoxikologie; Sicherheitspharmakologie	**category 9** biochemical toxikology; short time cancerogenicity; immunotoxicity; safety pharmacology
Datum der Inspektion 01.Sept.2008 bis 05.Sept.2008	**Date of Inspection** September 1st 2008 until September 5th 2008

Die/Der genannte Prüfeinrichtung/Prüfstandort befindet sich im nationalen GLP-Überwachungsverfahren und wird regelmäßig auf Einhaltung der GLP-Grundsätze überwacht.

Auf der Grundlage des Inspektionsberichtes wird hiermit bestätigt, dass in dieser Prüfeinrichtung/diesem Prüfstandort die oben genannten Prüfungen unter Einhaltung der GLP-Grundsätze durchgeführt werden können.

Düsseldorf, den 09.02.2009
Im Auftrag

(Dr. Deden)

The above mentioned test facility/ test site is included in the national GLP Compliance Programme and is inspected on a regular basis.

Based on the inspection report it can be confirmed, that this test facility/test site is able to conduct the aforementioned studies in compliance with the Principles of GLP.

Dienstsiegel/official-seal

11. Abbreviations used in the Tables

M	mean
SD	standard deviation
-S9	without S9 mix
+S9	with S9 mix
%	not tested
na	not assessable
P	precipitate
B	background lawn reduced
C	contaminated
AP	colonies visible but not countable
*	mutagenic effect
**	bacteriotoxic effect

12. Tables 1-10

Table 1

Bayer Schering Pharma
GDD-GED Toxicology
Genetic Toxicology
Wuppertal
Plate Test with : PES Vorstufe 2342

Study Number : T 5081015
Study Director : M. Nern
Technician : Boenning
Date : 28 FEB 2011
Strain: S. typhimurium TA 1535

Dose/Plate (µg/Plate)	Revertants per Plate -S9	M	SD	10 % +S9	M	SD	Titer Dilution 10^{-6}	Titer per ml 10^{+8}	Quotient -S9	Quotient +S9
DMSO	8 9 9	9	1	8 14 11	11	3	102 77	8.9	1.0	1.0
50	8 7 6	7	1	9 15 9	11	3	%	/	0.8	1.0
158	7 8 7	7	1	8 13 7	9	3	%	/	0.8	0.8
500	8 6 7	7	1	9 10 10	10	1	%	/	0.8	0.9
1581	7 P 8 P 8 P	8	1	8 P 11 P 7 P	9	2	%	/	0.9	0.8
5000	7 P 8 P 8 P	8	1	8 P 9 P 8 P	8	1	%	/	0.9	0.7
Na-azide 10	903 835 838	859	38	%	/	/	%	/	95.4 *	/
2-AA 3	%	/	/	112 118 125	118	7	%	/	/	10.7 *

Table 2

Bayer Schering Pharma
GDD-GED Toxicology
Genetic Toxicology
Wuppertal
Plate Test with : PES Vorstufe 2342

Study Number : T 5081015
Study Director : M. Nern
Technician : Boenning
Date : 28 FEB 2011
Strain: S. typhimurium TA 100

Dose/Plate (µg/Plate)	Revertants per Plate -S9	M	SD	10 % +S9	M	SD	Titer Dilution 10^{-6}	Titer per ml 10^{+8}	Quotient -S9	Quotient +S9
DMSO	165 154 135	151	15	205 217 194	205	12	140 128	13.4	1.0	1.0
50	161 150 124	145	19	191 197 217	202	14	%	/	1.0	1.0
158	165 130 150	148	18	183 186 213	194	17	%	/	1.0	0.9
500	135 167 153	152	16	206 217 181	201	18	%	/	1.0	1.0
1581	125 P 122 P 129 P	125	4	207 P 207 P 192 P	202	9	%	/	0.8	1.0
5000	144 P 149 P 135 P	143	7	194 P 213 P 220 P	209	13	%	/	0.9	1.0
NF 0.2	478 444 451	458	18	%	/	/	%	/	3.0 *	/
2-AA 3	%	/	/	3487 3124 3269	3293	183	%	/	/	16.1 *

Table 3

Bayer Schering Pharma
GDD-GED Toxicology
Genetic Toxicology
Wuppertal
Plate Test with : PES Vorstufe 2342

Study Number : T 5081015
Study Director : M. Nern
Technician : Boenning
Date : 28 FEB 2011
Strain: S. typhimurium TA 1537

Dose/Plate (µg/Plate)	Revertants per Plate -S9	M	SD	10 % +S9	M	SD	Titer Dilution 10^{-6}	Titer per ml 10^{+8}	Quotient -S9	Quotient +S9
DMSO	6 6 5	6	1	10 11 10	10	1	86 77	8.2	1.0	1.0
50	5 4 5	5	1	8 10 10	9	1	%	/	0.8	0.9
158	7 5 5	6	1	10 7 8	8	2	%	/	1.0	0.8
500	5 5 4	5	1	10 9 8	9	1	%	/	0.8	0.9
1581	4 P 6 P 4 P	5	1	8 P 8 P 7 P	8	1	%	/	0.8	0.8
5000	5 P 6 P 5 P	5	1	7 P 10 P 8 P	8	2	%	/	0.8	0.8
4-NPDA 10	35 41 37	38	3	%	/	/	%	/	6.3 *	/
2-AA 3	%	/	/	447 438 391	425	30	%	/	/	42.5 *

Table 4

Bayer Schering Pharma
GDD-GED Toxicology
Genetic Toxicology
Wuppertal
Plate Test with : PES Vorstufe 2342

Study Number : T 5081015
Study Director : M. Nern
Technician : Boenning
Date : 28 FEB 2011
Strain: S. typhimurium TA 98

Dose/Plate (µg/Plate)	Revertants per Plate -S9	M	SD	10 % +S9	M	SD	Titer Dilution 10^{-6}	Titer per ml 10^{+8}	Quotient -S9	Quotient +S9
DMSO	22 23 23	23	1	25 29 26	27	2	248 251	25.0	1.0	1.0
50	24 28 21	24	4	29 27 32	29	3	%	/	1.0	1.1
158	22 24 26	24	2	24 26 25	25	1	%	/	1.0	0.9
500	15 26 22	21	6	29 24 24	26	3	%	/	0.9	1.0
1581	24 P 20 P 20 P	21	2	27 P 22 P 30 P	26	4	%	/	0.9	1.0
5000	18 P 21 P 27 P	22	5	26 P 22 P 18 P	22	4	%	/	1.0	0.8
4-NPDA 0.5	82 95 75	84	10	%	/	/	%	/	3.7 *	/
2-AA 3	%	/	/	2418 2378 2136	2311	153	%	/	/	85.6 *

Table 5

Bayer Schering Pharma
GDD-GED Toxicology
Genetic Toxicology
Wuppertal
Plate Test with : PES Vorstufe 2342

Study Number : T 5081015
Study Director : M. Nern
Technician : Boenning
Date : 28 FEB 2011
Strain: S. typhimurium TA 102

Dose/Plate (µg/Plate)	Revertants per Plate -S9	M	SD	10 % +S9	M	SD	Titer Dilution 10^{-6}	Titer per ml 10^{+8}	Quotient -S9	Quotient +S9
DMSO	233 271 254	253	19	320 325 311	319	7	173 166	17.0	1.0	1.0
50	249 196 238	228	28	299 367 302	323	38	%	/	0.9	1.0
158	208 234 204	215	16	293 325 280	299	23	%	/	0.8	0.9
500	249 260 261	257	7	262 260 293	272	19	%	/	1.0	0.9
1581	216 P 243 P 216 P	225	16	286 P 262 P 269 P	272	12	%	/	0.9	0.9
5000	277 P 232 P 221 P	243	30	294 P 307 P 276 P	292	16	%	/	1.0	0.9
MMC 0.2	868 650 593	704	145	%	/	/	%	/	2.8 *	/
2-AA 3	%	/	/	780 875 808	821	49	%	/	/	2.6 *

Table 6

Bayer Schering Pharma
GDD-GED Toxicology
Genetic Toxicology
Wuppertal
Pre-Incubation with : PES Vorstufe 2342

Study Number : T 5081015
Study Director : M. Nern
Technician : Boenning
Date : 14 MAR 2011
Strain: S. typhimurium TA 1535

Dose/Tube (µg/Tube)	Revertants per Plate -S9	M	SD	10 % +S9		M	SD	Titer Dilution 10^{-6}	Titer per ml 10^{+8}	Quotient -S9	Quotient +S9
DMSO	10	9	1	8		9	1	85	8.5	1.0	1.0
	9			10				85			
	8			8							
50	9	8	1	9		9	0	%	/	0.9	1.0
	7			9							
	9			9							
158	7	8	1	7		8	2	%	/	0.9	0.9
	8			10							
	8			8							
500	9	9	1	7		8	1	%	/	1.0	0.9
	10			9							
	8			7							
1581	11	8	2	9		8	1	%	/	0.9	0.9
	7			7							
	7			9							
5000	7	6	1	8	P	10	2	%	/	0.7	1.1
	7			10	P						
	5			11	P						
Na-azide 10	846	818	30	%		/	/	%	/	90.9 *	/
	820										
	787										
2-AA 3	%	/	/	136		122	14	%	/	/	13.6 *
				122							
				108							

Table 7

Bayer Schering Pharma
GDD-GED Toxicology
Genetic Toxicology
Wuppertal
Pre-Incubation with : PES Vorstufe 2342

Study Number : T 5081015
Study Director : M. Nern
Technician : Boenning
Date : 14 MAR 2011
Strain: S. typhimurium TA 100

Dose/Tube (µg/Tube)	Revertants per Plate -S9	M	SD	10 % +S9		M	SD	Titer Dilution 10^{-6}	Titer per ml 10^{+8}	Quotient -S9	Quotient +S9
DMSO	148 111 127	129	19	181 205 193		193	12	151 90	12.1	1.0	1.0
50	136 135 144	138	5	197 186 193		192	6	%	/	1.1	1.0
158	137 119 130	129	9	161 188 172		174	14	%	/	1.0	0.9
500	141 116 151	136	18	175 177 206		186	17	%	/	1.1	1.0
1581	126 120 122	123	3	172 161 172		168	6	%	/	1.0	0.9
5000	114 126 124	121	6	144 144 146	P P P	145	1	%	/	0.9	0.8
NF 0.2	496 395 462	451	51	%		/	/	%	/	3.5 *	/
2-AA 3	%	/	/	2358 2176 2152		2229	113	%	/	/	11.5 *

Table 8

Bayer Schering Pharma
GDD-GED Toxicology
Genetic Toxicology
Wuppertal
Pre-Incubation with : PES Vorstufe 2342

Study Number : T 5081015
Study Director : M. Nern
Technician : Boenning
Date : 14 MAR 2011
Strain: S. typhimurium TA 1537

Dose/Tube (µg/Tube)	Revertants per Plate -S9	M	SD	10 % +S9		M	SD	Titer Dilution 10^{-6}	Titer per ml 10^{+8}	Quotient -S9	Quotient +S9	
DMSO	6	7	1	11		10	3	46	4.2	1.0	1.0	
	7			7				38				
	7			13								
50	6	7	1	11		9	2	%	/	1.0	0.9	
	8			7								
	7			9								
158	9	7	2	8		10	2	%	/	1.0	1.0	
	5			11								
	7			12								
500	7	6	1	12		10	2	%	/	0.9	1.0	
	6			8								
	5			11								
1581	6	5	1	7		8	1	%	/	0.7	0.8	
	4			8								
	6			8								
5000	4	4	1	8	P	7	1	%	/	0.6	0.7	
	5			7	P							
	4			7	P							
4-NPDA 10	35	33	3	%		/	/	%	/	4.7	/	*
	29											
	35											
2-AA 3	%	/	/	233		218	22	%	/	/	21.8	*
				229								
				193								

Table 9

Bayer Schering Pharma
GDD-GED Toxicology
Genetic Toxicology
Wuppertal
Pre-Incubation with : PES Vorstufe 2342

Study Number : T 5081015
Study Director : M. Nern
Technician : Boenning
Date : 14 MAR 2011
Strain: S. typhimurium TA 98

Dose/Tube (µg/Tube)	Revertants per Plate -S9	M	SD	10 % +S9		M	SD	Titer Dilution 10^{-6}	Titer per ml 10^{+8}	Quotient -S9	Quotient +S9
DMSO	13	15	3	17		20	2	71	7.4	1.0	1.0
	15			21				77			
	18			21							
50	17	15	2	21		18	3	%	/	1.0	0.9
	14			15							
	14			19							
158	18	16	2	22		22	1	%	/	1.1	1.1
	14			21							
	17			22							
500	15	13	3	24		25	1	%	/	0.9	1.3
	15			26							
	10			26							
1581	14	17	3	23		24	1	%	/	1.1	1.2
	19			24							
	19			24							
5000	16	15	2	16	P	20	5	%	/	1.0	1.0
	13			25	P						
	15			19	P						
4-NPDA 0.5	76	67	9	%		/	/	%	/	4.5 *	/
	66										
	59										
2-AA 3	%	/	/	1424		1492	72	%	/	/	74.6 *
				1484							
				1567							

Table 10

Bayer Schering Pharma
GDD-GED Toxicology
Genetic Toxicology
Wuppertal
Pre-Incubation with : PES Vorstufe 2342

Study Number : T 5081015
Study Director : M. Nern
Technician : Boenning
Date : 14 MAR 2011
Strain: S. typhimurium TA 102

Dose/Tube (µg/Tube)	Revertants per Plate -S9	M	SD	10 % +S9		M	SD	Titer Dilution 10^{-6}	per ml 10^{+8}	Quotient -S9	+S9
DMSO	268	282	13	327		314	12	165	16.3	1.0	1.0
	293			304				161			
	285			310							
50	287	273	16	345		339	7	%	/	1.0	1.1
	277			331							
	255			341							
158	289	262	25	380		349	36	%	/	0.9	1.1
	241			309							
	256			357							
500	286	291	19	351		327	35	%	/	1.0	1.0
	312			287							
	275			343							
1581	259	230	26	353		335	22	%	/	0.8	1.1
	218			310							
	212			341							
5000	240	248	23	321	P	294	24	%	/	0.9	0.9
	230			275	P						
	273			286	P						
Cumene 50	508	520	17	%		/	/	%	/	1.8 *	/
	539										
	512										
2-AA 3	%	/	/	641		610	32	%	/	/	1.9 *
				611							
				577							